The pineal gland, or epiphysis, sits on the roof of the diencephalon and is a simple photoreceptive structure in most anamniotes that acquired aneuroendocrine role in mammals. Its structure is comparable to the primitive eye found in mollusk or ascidians. Our fate map of the anterior neural plate, in zebrafish, localized the pineal precursors in a very lateral position, coinciding with the pre-placodal territory. Genetic loss of function studies further show that the formation of the epiphysis requires Dlx3, a protein strictly expressed in placodal ectoderm. Mis-expression experiments demonstrate that the combinatorial expression of the homeodomain transcription factors Dlx3 and Flh/Noto is sufficient to confer pineal identity. Finally, observations made in *otx1*;*otx2* double loss of function embryos reveal that pineal cell fate specification, controlled by Dlx3 and Noto, occurs during gastrulation and is negatively regulated by the Fgf signaling pathway. All together, our study uncovers the placodal nature of the pineal complex and identifies the genetic trigger of its specification during late gastrulation. Implications on evolution of the anterior neural ectoderm will be discussed.

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Program/Abstract # 415
Retinoic acid signaling plays key roles in the establishment of proximo-distal nephron segments in the zebrafish kidney
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The nephron is the basic functional unit of the kidney and is comprised of specialized segments of epithelial cells. How these segments form during nephrogenesis remains poorly understood. The zebrafish pronephros consists of two nephrons that arise from bilateral fields of intermediate mesoderm (IM). We have demonstrated that these nephrons are organized into proximal and distal segments, like mammals, and that retinoic acid (RA) plays an early role in defining proximal versus distal IM progenitors. This work establishes a new paradigm for the anatomy of the pronephros. We performed a forward genetic screen to isolate zebrafish with kidney segment defects, and isolated the *lightbulb* (*lib*) mutant that displays expanded distal segments and reduced proximal segments. This phenotype is strikingly reminiscent of embryos that lack RA. The distal expansion/proximal reduction of nephron segments in *lib* embryos is more severe than *raldh2*-deficient neckless mutant embryos, but less severe than DEAB-treated wild-types. Based on these findings, we hypothesized that *lib* mutants are RA-deficient. In support of this, *lib* can be rescued by treatment with exogenous RA. These data suggest that the *lib* gene product is essential for RA bioavailability, and provide further genetic support for the notion that RA signaling is integral for establishing the proximo-distal fates within the IM field. Continued study of *lib*, and the identification of the defective gene, will likely provide new insights into the mechanism by which RA patterns the IM into distinct renal progenitors, and further our understanding of nephron formation during embryogenesis.

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Program/Abstract # 416
Embryonic requirement for erbb signaling during zebrafish adult pigment pattern development
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Pigment cells and the patterns they form are a useful model for understanding the development of post-embryonic neural crest-derived lineages. Zebrafish pigment patterns undergo changes from a simple embryonic pattern to a more complex adult pattern during the period called metamorphosis, between two and four weeks post-fertilization. During this period, embryonic melanophores die and are replaced by new melanophores that differentiate from latent precursors and then they organize to form a different adult pigment pattern. We are using one class of mutant that has normal embryonic/early larval pigment patterns, but defective adult pigment patterns, to identify factors required to establish, maintain and recruit melanophore precursors during post-embryonic stages. picasso, which results from mutations in erbb3b, an epidermal growth factor receptor-like tyrosine kinase, is one such mutant. By means of morpholino and pharmacological inhibition with AG1478 and PD 158780, we identify a critical period during embryonic stages for erbb signals in promoting much later pigment pattern formation, with a peak sensitivity during neural crest cell migration. Using sensitized backgrounds, we also find cryptic requirement for erbb signaling during metamorphosis. Cell transplantation experiments indicate erbb3b activities that are both autonomous and non-autonomous to the melanophore lineage. These data are the first to identify a requirement for ErbB receptors in promoting pigment cell development and further suggest that erbb3b is required early to establish a population of adult pigment cell precursors that may be stem cells.

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Program/Abstract # 417
Isolation of a novel recessive maternal-effect dorsalizing mutation that expands the organizer
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During zebrafish embryogenesis dorsal–ventral patterning is achieved by mutual antagonism of ventralizing BMP signaling and dorsal fate specification mediated by the Spemann organizer. Mutations affecting BMP signal transduction lead to loss of ventral fates without expansion of the organizer. We isolated a novel recessive maternal-effect dorsalizing mutation, *p18ahub*, causing expansion of the organizer in a forward genetic screen for maternal factors required for zebrafish development. Although induction of the organizer at mid-blastula stages appears normal, *p18ahub* embryos have expanded axial mesoderm by late blastula stages and ectopic partial axes by late gastrulation. In contrast to all reported mutations affecting BMP signaling, *bmp* gene expression is reduced or absent from blastula stages throughout development in the most severely affected embryos. Such embryos are similar to radially dorsoanteriorized embryos that have been treated with lithium chloride, an inhibitor of Beta-Catenin degradation. The expression of *vox*, a repressor of dorsalizing genes, is also severely reduced or absent from blastula stages onwards. Based on their dorsal–ventral patterning defects, *p18ahub* embryos most resemble *wnt8* and *bmp2b* or *vox*, *vent*, and *ved* deficient embryos. Positional cloning of the mutant gene is underway. Future molecular-genetic studies of this mutant gene will likely further elucidate how ventralizing gene regulatory networks are integrated during vertebrate dorsal-ventral patterning.

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